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Activation of RecF-Dependent Recombination in *Escherichia coli* by Bacteriophage λ- and P22-Encoded Functions

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Escherichia coli **strains bearing wild-type and mutant alleles of various recombination genes, as well as plasmids that express recombination-related genes of bacteriophages λ and P22, were tested for their proficiency as recipients in Hfr-mediated conjugation. It was found that the homologous recombination systems of both phages could promote recombination in a *recB recC* mutant host. In addition, the Abc function of P22, but not the Gam function of λ, was found to inhibit recombination in a wild-type host; however, both Abc and Gam inhibited recombination in a *recF* mutant host. These observations are interpreted as indicating that the recombination systems of both phages, as well as the RecBCD-modulating functions Abc and Gam, all activate the RecF recombination pathway of *E. coli*.**

Bacteriophages λ and P22 encode their own homologous recombination systems, which promote recombination between phage chromosomes even in the absence of host-encoded RecA and RecBCD proteins (2, 4, 5, 13–15). This observation raises the question of whether the phage systems can promote other recombination events. Weisberg and Sternberg (18) found that expression of the λ *red* genes in infected *recB* mutant cells could promote efficient λ-mediated specialized transduction; ordinarily, such strains are defective in this process. The *red* system did not complement the deficiency of *recA* or *recA recB* mutants in these studies. We sought to examine further the interactions of phage- and host-encoded recombination systems by using plasmids that express phage recombination genes in the absence of other phage genes.

In the experiments summarized in Table 1, wild-type and mutant strains of *Escherichia coli* bearing plasmids that express various λ and P22 genes were tested for their abilities to serve as recipients in Hfr-mediated conjugation. In the construction of these plasmids, described previously (5, 11, 12), phage genes were fused to the *lacUV5* promoter and ligated into the *lacI*-bearing, tetracycline resistance-conferring vector pMC7. Effective expression of the phage genes was induced by adding isopropyl-β-D-thiogalactopyranoside (IPTG) to the medium.

Recipient strains were grown by being rolled in culture tubes at 37°C in L broth (supplemented with 4 to 5 μg of tetracycline per ml for plasmid-bearing cells) to a density of 60 to 100 Klett units. Cultures were diluted with L broth to equalize densities (25 to 30 Klett units in different experiments) and to reduce the tetracycline concentration in all cases to 2 μg/ml. (It was found that tetracycline at this concentration had no effect on the mating proficiency of the tetracycline-sensitive donor [data not shown].) IPTG was added to the indicated concentration, and rolling was resumed at 37°C for 65 to 75 min. Matings were carried out by mixing 0.2 ml of recipient culture with 0.04 ml of a donor culture grown in a shallow layer of L broth without shaking at 37°C to a density of 45 Klett units; the mixtures were incubated without shaking at 37°C for 45 min. Mating pairs were disrupted by diluting and vortexing in buffer E (16). In experiments 1, 3, and 4, the donor was BW113 (Hfr PO3 *metB1 relA1 spoT1*). Recipient titers were determined by plating the mating mixtures on minimal medium containing E buffer, glucose (0.4%), Casamino Acid hydrolysate (0.2%), thiamine (0.2 μg/ml), and streptomycin (100 μg/ml). Donor titers were determined by mixing cells with the appropriate volume of L broth, incubating in parallel with the mating mixtures, and plating on L agar. Recombinant titers were determined by plating the mating mixtures on minimal E glucose agar plates containing streptomycin, arginine, histidine, and proline (100 μg/ml each). In experiment 2, the donor was MV1955 (a *thr-35*::Tn*9* derivative of BW113). Donor titers were determined as in the other experiments except that the plating medium was L agar containing 25 μg of chloramphenicol per ml. Recipient titers were determined by plating the mating mixtures on L agar containing 100 μg of streptomycin per ml. Recombinant titers were determined by plating the mating mixtures on L agar containing both chloramphenicol and streptomycin. In all experiments, titers for control mixtures that lacked either donors or recipients were determined under selective conditions; the background was undetectable in all cases (data not shown).

Table 1 (experiment 1) shows that plasmids expressing the recombination systems of either P22 (*erf*, *abc*1, and *abc*2) or λ (the *red* genes, *exo* and *bet*) promote the formation of recombinants in a *recB recC* mutant host. The effect is partially dependent on the addition of IPTG to the medium (presumably, the non-IPTG-dependent part of the effect is due to the nonzero basal expression of the plasmid-borne recombination genes). Neither the vector plasmid nor IPTG itself had a significant effect in this test. The magnitude of the stimulation by λ *red* was approximately 40-fold, bringing the level of recombination in the *recBC* mutant host up to as much as 11% of the wild-type frequency. The experiment shown is typical in this regard. In five independent measurements, the range of stimulation was 29- to 85-fold (data not shown). In this test, the P22 system was a little more than half as active as the λ system. In addition to the plasmids listed, we tested plasmids that express *bet*, *exo*, *erf*, or *abc*1 and *abc*2; none had significant activity, indicating that more than one component of each system is required for recombinant formation to be stimulated (data not shown).

Table 2 (experiment 2) shows that recombination in a *recB recC* host bearing a *red*-expressing plasmid is dependent on both *recA* and *recF* (or *sbcB*). The test differed somewhat from that in experiment 1, in which streptomycin-resistant recombinants that inherited the *thr*$^+$ and *leu*$^+$ alleles of the

TABLE 1. Recombination in Hfr crosses

Recipient[a]		Function(s)	IPTG concn (mM)	% of wild-type recombination frequency[b]
Expt no. and host	Plasmid			
Expt 1				
recBC	None	None	0	0.25
			1.0	0.39
	pMC7	None	0	0.28
			1.0	0.26
	pTP178	*erf* and *abc*	0	4.1
			0.125	6.3
			0.25	5.8
			0.50	5.8
			1.0	5.8
	pTP232	*bet* and *exo*	0	0.42
			0.125	7.9
			0.25	11
			0.50	10
			1.0	11
Expt 2				
recBC	pMC7	None	1.0	0.20
	pTP232	*bet* and *exo*		6.4
recA recBC	pMC7	None		0.0055
	pTP232	*bet* and *exo*		0.003
recBC sbcB recF	pMC7	None		0.0086
	pTP232	*bet* and *exo*		0.0020
Expt 3				
Wild type	pMC7	None	1.0	59
	pTP224	*gam*		50
	pKM420	*abc*1		79
	pKM451	*abc*2		29
	pKM460	*abc*1 and *abc*2		8.8
Expt 4				
recF	None	None	1.0	78
	pMC7	None		44
	pTP224	*gam*		14
	pKM451	*abc*2		12
	pKM460	*abc*1 and *abc*2		2.6

[a] Host strains were all derivatives of *E. coli* K-12 strain AB1157 (F⁻ *argE3 his-4 leu-6 proA2 thr-1 ara-14 galK2 lacY1 mtl-1 xyl-5 thi-1 rpsL31 supE44 tsx-33*). JC5519 (*recB21 recC22*), JC5547 (*recA13 recB21 recC22*), JC8111 (*recB21 recC22 sbcB15 recF143*), and JC9239 (*recF143*) were from A. J. Clark. MV1161 (*rfa-550*) was the reference wild-type strain. Plasmids were described previously (5, 10, 11).

[b] Frequencies obtained with the wild-type recipient were 1.9, 2.2, 3.4, and 2.7 recombinants per 100 donors in experiments 1 to 4, respectively.

donor and the streptomycin resistance of the recipients were identified by their ability to grow on minimal medium containing streptomycin but lacking threonine and leucine. We found that *recA*, *recA recB recC*, and *recB recC sbcB recF* mutant strains bearing any of the plasmids indicated in Table 1 died when subjected to a nutritional downshift from rich medium, even when all of their known growth requirements were met. For this reason, we employed a nearly isogenic Hfr bearing a Tn*9* insertion in the *thr* gene and identified recombinants that had inherited the donor *thr-35*::Tn*9* allele by their ability to grow on rich medium that contained both streptomycin and chloramphenicol. As indicated, the *red*-expressing plasmid stimulated recombination in the *recB recC* host to approximately the same extent as in experiment 1. However, it did not stimulate recombination in either the *recA recB recC* strain or the *recB recC sbcB recF* strain. Similarly, it failed to promote recombination in a simple *recA* mutant (data not shown).

If the phage proteins could functionally replace RecBCD, we would expect that the recombination they promote to be independent of RecF (3). The results shown in Table 1 suggest that Red, rather than functionally replacing RecBCD, works by activating the RecF recombination pathway. This interpretation leads to the prediction that the resulting recombination would depend also on the other genes of the RecF pathway (17). We attempted to test this prediction in suitably configured *recJ* and *recQ* mutant strains, but introduction of our plasmids into these hosts made them grow so poorly that they were intractable.

The dependence of Red-promoted recombination on RecA in these tests is perplexing in light of the observation that Red-promoted recombination between phage chromosomes is independent of RecA (15). However, the DNA substrates for recombination in a λ-infected cell and in a recipient cell following conjugation differ substantially. In particular, the DNA transferred by the Hfr strain is nonreplicating, and the sequences at the transferred end have no homology to the recipient cell chromosome (they are part of the F plasmid). Stahl and co-workers (14) have shown that when λ DNA replication is blocked, Red promotes crossovers only very near ends generated by the phage DNA packaging apparatus cutting at *cos*. If Red works by inserting a DNA end into a homologous sequence in another chromosome, we would not expect it to promote recombination in our test.

Our finding of an apparent stimulation of the RecF recombination pathway by Red is consistent with studies by Armengod (1) of recombination between prophage λ and heteroimmune infecting λ. In these studies, it was found that such recombination depended on Red, RecA, and RecF when replication of the infecting phage was blocked.

In addition to elaborating their own recombination systems, λ and P22 both encode functions—Gam and Abc, respectively—that modulate the activities of the *E. coli* RecBCD protein (12, 20). Hays and co-workers (6, 8, 19) have found that Gam has little effect on recombination in a wild-type cell following transduction or conjugation. Another study (12) detected inhibition by both Gam and Abc of host system-mediated recombination between λ chromosomes bearing chi sites. In an attempt to unify these observations, we examined the effects of plasmids that express *gam* and *abc* on recombination following conjugation in a wild-type host.

In experiment 3, a plasmid that expresses P22 genes *abc*1 and *abc*2 (10) significantly inhibited recombination in a wild-type host. Gam had no significant effect in this test, nor did Abc1 alone. Abc2 alone had a slight inhibitory effect.

In experiment 4, we sought to determine whether the residual recombination in a wild-type cell containing either Gam or Abc is dependent on RecF. As indicated, both Gam and Abc2 inhibited recombination in a *recF* mutant host more than 3-fold, while the combination of Abc1 and Abc2 inhibited recombination 17-fold. We interpret this result to mean that both Gam and Abc inhibit RecBCD-mediated recombination but at the same time stimulate the RecF pathway. As observed previously (12), the inhibitory effect of Abc is greater than that of Gam.

A question raised by the foregoing observations is whether stimulation of the RecF pathway by Gam and Abc occurs as a consequence of the action of these functions on RecBCD or on something else (such as ExoI, the product of the *sbcB* gene). If the latter were the case, we predict that Gam and Abc would actually stimulate recombination in a *recB recC* mutant. We were unable to detect such stimulation (data not shown); this finding leads us to prefer the former interpretation, i.e., that Gam and Abc stimulate the RecF pathway through their actions on RecBCD. It is known that Gam modulates RecBCD activity directly (9), and it is suspected that Abc does so as well (12). Note that stimulation of the RecF pathway by Gam and Abc differs from that mediated by the phage recombination systems in that the latter can be seen in a *recB recC* mutant host.

The restoration of recombination proficiency in *recB recC* mutant strains by *sbcA* mutations shares a number of features with the restoration that we observe following introduction of a λ *red*-expressing plasmid. In both cases, the resulting recombination is dependent on *recA*, *recF*, and a phage recombination function (either *red* or the *recE* gene borne by the cryptic Rac prophage; 7).

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